'Neuroendocrine peptide mechanisms controlling intestinal epithelial function'

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Highlights

- Dietary nutrients and microbial metabolites can selectively activate different EECs.
- L cell peptides, PYY and GLP-1 can be stored independently and released differentially.
- Selective GPR119, FFA1-4 and MC4R agonism cause L cell peptide release but,
- there are some notable species and intestinal area differences.
- Dual agonism amplifies incretin release and could be more physiologically relevant.

Abstract

Enteroendocrine cells (EECs) contain different combinations of hormones, which are released following stimulation of nutrient receptors that are selectively expressed by these cells. This chemosensation varies according to the intestinal area and species of interest, and responses to meals are rapidly modified following bariatric surgery. Such surgically-induced gastrointestinal (GI) changes highlight considerable enteroplasticity, however our understanding of even the acute physiological control and consequences of neuroendocrine peptide release is still under-developed. This review focuses on recent advances in nutrient G protein-coupled receptor (GPCR)-chemosensation in L cells, the patterns of peptide release and consequent changes in GI function. A clearer resolution of these mucosal mechanisms will shed light on potential receptor-target combinations that could provide less-invasive anti-diabesity strategies in future.

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Introduction

It is notable that certain bariatric surgeries have striking neuroendocrine effects that are implicated to contribute to weight loss and resolution of type 2 diabetes (T2D) [1]. Accelerated nutrient delivery to more distal intestinal regions where nutrient-sensitive, open-type EECs predominate (particularly L cells), leads to enhanced release of gastrointestinal (GI) hormones, such as peptide YY (PYY), glucagon-like peptide 1 (GLP-1), GLP-2, oxyntomodulin, and glucose-dependent insulinotropic peptide (GIP) [1] that are pro-absorptive, proliferative, facilitate insulin secretion and can suppress appetite. Mucosal hyperplasia and increased cellular metabolism also occur and this GI remodelling contributes to the therapeutic benefits of bypass surgery [2] but the underlying integrated changes in neuroendocrine signalling are inadequately understood. Variations in EEC developmental lineages are also apparent with likely physiological consequences [3,4,5].

Variations in peptide expression and release from L cells in different species and GI areas

EECs have for some time been known to extend elongated vesicle-filled basal processes under the basolateral surface, recent ultrastructural studies showing they can extend underneath 10-15 adjacent epithelial cells [6] implicating paracrine modulation of mucosal function. These basal processes appear to be guided by enteric glia towards submucosal enteric neurons, a proportion of which are calcitonin gene-related peptide (CGRP)-containing [7*] and potentially sensory in function. This connectivity complements that established using rotaviral and cholera toxin, both of which activate 5HT-containing enterochromaffin cells (ECs) resulting in enteric nerve stimulation [8,9]. Some L cells are long-lasting residents, remaining in the mucosa for up to 60 days [7*] about 10 times longer than their surrounding epithelial cells, indicating prolonged signaling role(s). The EEC capacity to mediate afferent signals from luminal nutrients is well established (having been shown for CCK cells, ghrelin cells, L cells and duodenal 5-HT cells), but the identification of specific enteric neurons involved is still in its infancy.

L cell frequency increases distally in most mammalian intestines but the gradient varies between species e.g. it is steeper in the mouse compared with the pig GI tract [10,11]. L cell peptide combinations also vary along the gut length [4,5,10] with a greater peptide number exhibited in small intestinal mucosa [4,5]. Different L cell subtypes result from variations in the numbers of peptides per cell; proximal L cells containing GIP and CCK, both peptides more typical of K- and I-cells [10,12], plus neurotensin [4,5]. The peptide repertoire also changes as cells progress from crypt to villus. Crypt GLP-1 cells appear to gain peptide partners as they travel towards the lumen [13*] and such plasticity is likely to have functional consequences. In the mouse distal ileum neurotensin is co-released with PYY and GLP-1 causing synergistic reductions in gastric emptying and food intake, despite a degree of separate vesicle packaging [13*]. Super-resolution confocal microscopy has shown that PYY and GLP-1 are often segregated in different vesicle populations of mouse, pig, rat and human L cells [14*] providing the possibility for differential peptide release. Indeed, this may occur in response to the luminal microbiota-generated short chain fatty acid (SCFA) propionate; PYY levels far exceeded those of GLP-1, while GIP appearance did not change significantly (in rat small intestine) [15]. Hormonal release from K- and L cells is glucose-dependent, involving sodium-dependent glucose co-transporter (SGLT1) and GLUT2 [15,16] confirmed by functional studies (below). However, further *in vivo* studies are required to better resolve the patterns and time-dependence of peptide co-release linking them with GI functions. In summary, EEC peptide expression patterns change along the crypt-villus axis, the gut length and between species and some L cells are much longer-lived than their epithelial neighbours [7*]. This remarkable variability and plasticity very likely underpins both the amplified hormone responses seen after bariatric surgery and the loss of absorptive capacity when EEC numbers are depleted or their hormone processing is incomplete [1,2,3].

Nutrient-sensing, MC4R signaling, and microbial metabolite-sensing pathways involving L cells

The generation of reporter mice has provided impetus and superior characterisation of EECs [16,17]. The proglucagon promoter-driven transgenic mouse confirmed the predominance of L cells in colonic regions and identified gluco-sensory machinery (SGLT1, glucokinase) and K_{ATP} channel subunits selectively in L cells [18]. The oleoylethanolamide (OEA) and 2-monoacyl glycerol (2-MAG) lipid sensor GPR119, plus GPR40 (now known as free fatty acid receptor 1, FFA1), GPR120 (free fatty acid receptor 4, FFA4) and the bile acid receptor, TGR5 (also known as G protein-coupled bile acid receptor 1, GPBAR1) were discovered to be highly L cell-expressed [18]. A similar range of glucose-, and fat-sensing receptors (GPR119, FFA1 and FFA4) were identified in K cells from GIP promotor-driven transgenic mice, while low levels of sweet tastant receptors were observed, only in colonic L cells [19]. Readers are directed elsewhere for information on sweet and other taste receptor signalling (e.g. T1R2-T1R3; Figure 1) [20].

The first description that GPR119 immunolabelling was limited to L cells along the length of human and mouse intestine as well as pancreatic β cells [21] remains notable. Selective GPR119 agonism increased both GIP and GLP-1 release and improved glycaemic control in wild type (WT) but not GPR119 knockout (KO) mice [21]. This inspired the investigation of small molecule GPR119 agonists, OEA and other lipids with GPR119 affinity, in mucosal preparations from which the epithelial ion transport effects of endogenous peptide release could be measured [22]. GPR119 responses were more pronounced in the distal colon, matching L cell predominance and PYY-Y1 receptor distribution (Figure 1). Symonds *et al*. [23**] observed increasing incidence of GPR119 (also FFA2 and FFA4) expression down the mouse gut. In contrast, consistent levels of TGR5, the protein hydrolysate receptor (GPR93), amino acid/calcium-sensing receptor (CaSR) and fatty acid receptors (FFA1, FFA3 and GPR84 a lauric acid-sensitive receptor) were observed. In man, the only notable increase from antrum-to-rectum was for FFA4 expression [23**]. A notable species difference shows CaSR expression exclusively in mouse L cells, while human colonic L cells and 5HT-containing ECs both express CaSR [23**] and the functional consequence of this difference has yet to be determined.

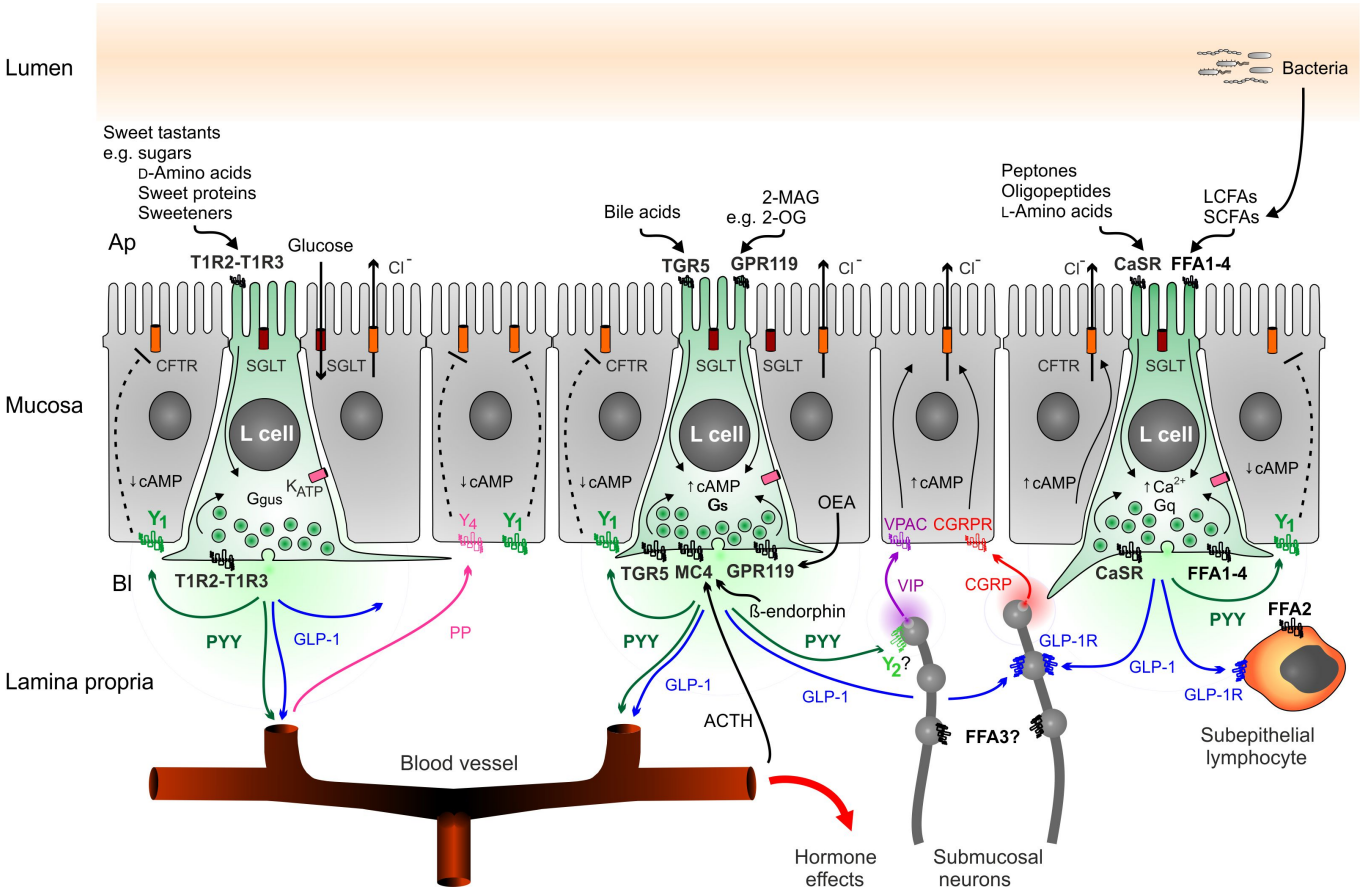

Figure 1. Schematic of GPCR-induced L cell signaling and consequent paracrine peptide mechanisms in mouse and human colon mucosa. For clarity L cells with gustducin-, G_s- or G_q-coupled signaling are drawn separately, although the pathways overlap. Apical (Ap) sweet taste receptors (e.g. T1R2-T1R3 heterodimers) sense luminal sugars and can release PYY and GLP-1. Ap or basolateral (Bl) GPR119 are stimulated either by luminal nutrients (e.g. 2-MAG such as 2-OG) or endogenous lipids (e.g. OEA). Basolaterally-targeted MC4 receptors are most likely activated by the hormone ACTH and/or locally released β-endorphin (possibly from tuft cells, not shown). These G_s-coupled pathways result in predominant PYY (over GLP-1) signaling via epithelial G_i-coupled Y_1 receptors, reduced intra-epithelial cAMP levels and attenuated Cl^- transport through apical epithelial Cl^- channels (CFTR). Presynaptic Y_2 receptors mediate a proportion of endogenous PYY response via submucosal neuron(s) whose identity is not yet known (they may be vasoactive intestinal polypeptide (VIP) neurons). When GLP-1 is involved it acts on GLP-1R, and in the colon these receptors are present on CGRP-containing neurons; the neuropeptide then activates epithelial CGRPR. The GLP-1R is additionally expressed by intra- and subepithelial lymphocytes, which also express FFA2. For G_q-coupled L cell mechanisms all four FFA receptors (FFA1-4) and CaSR are probably present on both Ap and Bl domains and activated by metabolites in the lumen or lamina propria. Very few studies include oxyntomodulin's roles so this L cell peptide is omitted for now.

GPR119 and the melanocortin 4 receptor (MC4R; better known as part of the central homeostatic response to dietary fats) are both G_s-coupled and amongst the most highly expressed L cell receptors [24*, Schwartz *et al.* unpublished]. Agonism promotes PYY and GLP-1 release with consequent clear PYY-Y1 receptor (Y1R) epithelial anti-secretory and incretin effects [22, 24*]. Both receptor activities are glucose-dependent and their peptide mediator pharmacologies are the same in mouse and human mucosae (Figure 1). Epithelial Y1R and Y4R, and neural Y2R mechanisms mediate the full repertoire of PYY-related (including neuropeptide Y, NPY and pancreatic polypeptide, PP) electrogenic responses in mouse and human mucosae [25,26,27,28] but there are significant differences between these two species and the rat. In the latter, Y2R are epithelial while Y1R are pre- and post-junctional in colon [29] and MC4R activation has no effect on PYY or GLP-1 release [24*] in stark contrast to mouse and human mucosal responses. GPR119 stimulation does however cause PYY and GLP-1 release in rat intestine [13*,15] with consequent PYY-Y1R mucosal responses; activities that are also present in

models of T2D [30*]. Mucosal GLP-1R activity (in mouse colon) involves calcitonin gene-related peptide (CGRP) release from enteric submucosal neurons [31] as implicated by the close apposition of L cells and GLP-1R positive nerves [32*] (Figure 1). Hormonal PYY is well established as a mediator of postprandial ileal brake which together with the Y2R-prefering product, PYY(3-36) and GLP-1 retards GI transit [24*,28,33,34], these peripheral mechanisms contributing to the lean phenotype.

GPR119 specific agonists include the digestive products of dietary triacylglycerol (2-MAG; e.g. 2-OG) as likely luminal stimulants [35] alongside endogenous OEA and other oleic acid-containing lipids [36]. GPR119 is located on both luminal and basal surfaces (Schwartz, Cox *et al*. unpublished) and is therefore accessible to exogenous and endogenous lipids. The stress hormone ACTH, and β-endorphin (derived from tuft/brush cells or neurons) are both potential MC4R agonists, while a gut bacterial mimetic of αMSH has been proposed recently as a MC4R activator [37*] although, how this protein accesses the basolaterally-located MC4R is unclear. Notably, GPR119 and MC4R tonic activities exist in the colon and when blocked selectively result in similar epithelial responses and faster colonic transit [22,24] implicating ongoing anti-secretory and anti-motility effects of L cell PYY. Conditional GPR119 knockout from proglucagon-expressing α- and L cells leads to impaired lipid-stimulated GLP-1 release *in vivo* and reduced GPR119 agonism *in vitro* [38]. Acute loss of α- and L cells also impairs glucose metabolism [39], further highlighting their physiological importance.

The four free fatty acid receptors FFA1-4, are also expressed by EECs but not surrounding epithelial cells, in mouse and human intestine [18,19,23] with FFA4 being more prominent in colonic mucosa [23**,40]. Activation of FFA1 and FFA4 requires FA (saturated or unsaturated) of C12-C22 chain lengths e.g. palmitic or oleic acids for FFA1, ω3 fatty acids for FFA4 [40,41]. K cells also express FFA1 and FFA4 [19], GLP-1 and GIP secretion occurring upon activation by selective ligands or dietary FAs [42,43]. Use of synthetic FFA1 agonists indicates (in rat small intestine) that this receptor maybe preferentially targeted to basolateral surfaces [44]. However, these lipid soluble agents preclude absolute sidedness being determined. The G_q-signalling pathway most likely mediates FFA1 and FFA4-induced GLP-1, PYY and insulin secretion, but Hauge *et al*. [45*] recently described a FFA1 agonist that co-activates G_s- and G_q-pathways with significantly elevated GLP-1 secretory capacity *in vitro* and *in vivo*. Additionally, α-gustducin can mediate some FFA1-4, GPR119- and TGR5-induced GLP-1 release in colonic mucosa [46] and apical tastant receptor activation causes PYY-Y1 responses [47] and GLP-1 release (Figure 1). Dietary triglycerides (TG) are digested and sensed by EECs as FFAs with 2-MAG which activates G_s-coupled GPR119, so dual- (or more complex) agonism is more physiologically relevant and provides amplified incretin responses [45*]. In addition, micellar formation of dietary TGs with bile acids (BAs; that alone stimulate TGR5) could also co-activate mucosal pathways with enhanced efficacy. TGR5 is highly expressed in EECs [18,23] and BAs trigger GLP-1 [18,48], PYY and GIP secretion [15] with a degree of basolateral preference in mouse distal ileum [48,49] indicating that BAs may require absorption prior to chemosensation via TGR5. Interestingly, others have shown luminal BAs inhibit GI transit via neurogenic TGR5 mechanisms [50] while we observe epithelial responses to BAs added to either surface [Cox *et al*. unpublished]. Intestinal BA concentrations are influenced by dietary fat and protein, and are modified by the colonic microbes. These metabolites affect gut motility differently, unconjugated BAs correlate with faster whole-gut transit while conjugated BAs appear to be associated with slower transit [51**], aspects of which may also be mediated by the nuclear BA farsenoid X receptor, providing further functional complexity to diet-microbial-BA signalling.

SCFA receptors, FFA2 (GPR43) and to a lesser extent, FFA3 (GPR41) are expressed by L cells [23**, 52,53,54] (Figure 1). FFA2 is also found on subepithelial lymphocytes while FFA3 appears to be a reporter gene in most EECs and more notably in enteric neurons [53] although which neuron type(s) is unclear. The major products of bacterial fermentation of dietary fibre, i.e. luminal acetate, propionate and butyrate, are not only epithelial energy sources but they also stimulate PYY and GLP-1 release in rat, mouse and human small intestine [15,52,53,54]. Selective (G_q-linked) FFA2 agonism promotes robust PYY release with consequent PYY-Y1R epithelial responses, reduced transit and food intake, [55,56*] with minimal GLP-1 involvement even when dipeptidyl peptidase-4 is blocked [56*]. FFA2 activity encompasses GI proliferative and anti-inflammatory effects [57; part-mediated via butyrate-

preferring GPR109A; 58*], linking colonic microflora, FFA-signalling and host mucosal integrity and adaptive immunity. FFA3-mediated intestinal gluconeogenesis underpins the metabolic benefits of portal propionate [59**] while intracolonic propionate administration to mice [60] and humans [61*] results in PYY and GLP-1 release and longer-term prevention of weight gain in humans, further highlighting the therapeutic potential of SCFA-mimetics.

Glutamine is a supplement used in parenteral nutrition that also increases GLP-1, GIP, PYY, glucagon and insulin release in rodents and man [15,62,63]. These effects are mediated in part by CaSR, which is highly expressed by L cells resulting in the co-activation of G_q- and G_s-pathways *in vitro* [63]. Symonds *et al*. [23**] observed possible cell entrainment, groups of epithelial cells surrounding a CaSR-G_q-stimulated EEC were activated in mouse colon, but how prevalent this effect is has yet to be established. Functional studies do indicate that physiologically relevant levels of glutamine or other L-amino acids are effective on apical and basolateral surfaces [64] and these responses are glucose-dependent [65]. Oligopeptides and peptones also act via CaSR to release GLP-1 [15,66,67]. Thus, many products of dietary protein stimulate incretin release exerting acute paracrine epithelial effects, often slowing GI transit, improving glucose tolerance and/or reducing food intake.

Conclusions

The discovery that numerous nutrient GPCRs are expressed discretely by EECs, highlights their chemosensory roles. However, physiologically meaningful combinations of luminal and/or blood borne nutrients have yet to be compared in terms of their hormone releasing capacities. Knowledge of the interactions of nutrient combinations (usually encountered by EECs) will be fundamental to understanding the acute and longer-term modulatory effects of dietary constituents and endogenous ligands on EEC signalling linked to amplified incretin peptide secretion and reduced weight. L cell plasticity is clearly evident in disease, numbers decline in metabolic syndrome, T2D, or obesity, and increase after bariatric surgery and in chronic diarrhoea. In future targeted nutrient, or nutrient receptor agonist delivery to the distal bowel where higher levels of nutrient-sensitive GPCRs exist, may well recapitulate the effects of bariatric surgery, offering dietary-inducible, intestinal-limited mechanisms with anti-diabetic and anti-obesity potential.

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Conflict of interest

HC has no competing interests to declare.

References and recommended reading

Papers of particular interest have been highlighted as: * of special interest, ** of outstanding interest

1. Seeley RJ, Chambers AP, Sandoval DA: The role of gut adaptation in the potent effects of multiple bariatric surgeries on obesity and diabetes. *Cell Metab* 2015, **21:**369-378.

2. Saeidi N, Meoli L, Nestoridi E, Gupta NK, Kvas S, Kucharczyk J, Bonab AA, Fischman AJ, Yarmush ML, Stylopoulos N: Reprogramming of intestinal glucose metabolism and glycemic control in rats after gastric bypass. *Science.* 2013, **341**:406-410.

3. Drucker DJ: Evolving concepts and translational relevance of enteroendocrine cell biology. *J Clin Endocrinol Metab* 2016, 101:778-786.

4. Egerod KL, Engelstoft MS, Grunddal KV, Nohr MK, Secher A, Sakata I, Pedersen J, Windelov JA, Fuchtbauer EM, Olsen J *et al*.: A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1, PYY and neurotensin but not somatostatin. *Endocrinol* 2012, **153**:5782-5795.

5. Habib AM, Richards P, Cairns LS, Rogers GJ, Bannon CA, Parker HE, Morley TC, Yeo GS, Reimann F, Gribble FM: Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. *Endocrinol* 2012, **153**:3054-3065.

6. Bohorquez DV, Samsa LA, Roholt A, Medicetty S, Chandra R, Liddle RA: An enteroendocrine cell - enteric glia connection revealed by 3D electron microscopy. *PLOS One* 2014, **9**:e89881

7. * Bohorquez DV, Shahid RA, Erdmann A, Kreger AM, Wang Y, Calakos N, Wang F, Liddle RA: Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin Invest* 2015, **125:**782-786. A timely study showing PYY-containing EECs form synapses from their basal processes with efferent enteric (submucosal) neurons.

8. Lundgren O, Peregrin AT, Oersson K, Kordasti S, Uhnoo I, Svensson L: Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science* 2000, **287**:491-495.

9. Gwynne RM, Ellis M, Sjövall H, Bornstein JC: Cholera toxin induces sustained hyperexcitability in submucosal secretomotor neurons in guinea pig jejunum. *Gastroenterol* 2009, **136**:299-308.

10. Cho HJ, Kosari S, Hunne B, Callaghan B, Rivera LR, Bravo DM, Furness JB: Differences in hormone localisation patterns of K and L type enteroendocrine cells in the mouse and pig small intestine and colon. *Cell Tissue Res* 2015, **359**:693-698.

11. Wewer Albrechtsen NJ, Kuhre RE, Torang S, Holst JJ: The intestinal distribution pattern of appetite- and glucose regulatory peptides in mice, rats and pigs. *BMC Res Notes* 2016, **9**:60-64.

12. Sykaras AG, Demenis C, Cheng L, Pisitkun T, Mclaughlin JT, Fenton RA, Smith CP: Duodenal CCK cells from male mice express multiple hormones including ghrelin. *Diabetes* 2014, **155**:3339-3351.

13. * Grunddal KV, Ratner CF, Svendsen B, Sommer F, Engelstoft MS, Madsen AN, Pedersen J, Nøhr MK, Egerod KL, Nawrocki AR *et al*.: Neurotensin is co-expressed, co-released and acts together with GLP-1 and PYY in enteroendocrine control of metabolism. *Endocrinol* 2016, **157**:176-194. A study showing that in distal ileum L cells neurotensin is stored in separate vesicles from those containing GLP-1 and/or PYY. Neurotensin acts synergistically *in vivo* with GLP-1 and PYY to for example, decrease food intake and slow gastric emptying.

14. * Cho HJ, Robinson ES, Rivera LR, McMillan PJ, Testro A, Nikfarjam M, Bravo DM, Furness JB: Glucagon-like peptide 1 and peptide YY are in separate storage organelles in enteroendocrine cells. *Cell Tissue Res* 2014, **357**: 63-69. The first study showing segregated peptide packaging in EECs from rat, mouse, pig and human bowel.

15. Mace OJ, Schindler M, Patel S: The regulation of K- and L-cell activity by GLUT2 and the calcium-sensing receptor CasR in rat small intestine. *J Physiol* 2012, **590.12**:2917-2936.

16. Pais R, Gribble FM, Reimann F: Stimulation of incretin secreting cells. *Therap Adv Endocrinol & Metab* 2016, **7**:24-42.

17. Engelstoft MS, Egerod KL, Lund ML, Schwartz TW: Enteroendocrine cell types revisited. *Curr Opinions Pharmacol* 2013, **13**:912-921.

18. Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ, Gribble FM: Glucose sensing in L cells: a primary study. *Cell Metab* 2008, **8**:532-539.

19. Parker HE, Habib A, Rogers GJ, Gribble FM, Reimann F: Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia* 2009, **52**:289-298.

20. Efeyan A, Comb WC, Sabatini DM: Nutrient-sensing mechanisms and pathways. *Nature 2015*, **517**:302-310.

21. Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, Pedraza M, Mondala H, Gao H, Bagnol D *et al*.: A role for intestinal endocrine cell-expressed GPR119 in glycemic control by enhancing GLP-1 and GIP release. *Endocrinol* 2008, **149**:2038-2047.

22. Cox HM, Tough IR, Woolston AM, Zhang L, Nguyen AD, Sainsbury A, Herzog H: PYY is critical for acylethanolamine receptor Gpr119-induced activation of gastrointestinal mucosal responses. *Cell Metab* 2010, **11**:532-542.

23. ** Symonds EL, Peiris M, Page AJ, Chia B, Dogra H, Masding A, Galanakis V, Atiba M, Bulmer D, Young RL *et al*.: Mechanisms of activation of mouse and human enteroendocrine cells by nutrients. *Gut* 2015, **64**:618-626. An important paper describing graded expression of different nutrient GPCRs in L cells along the length of the mouse and human GI tract.

24. * Panaro BL, Tough IR, Engelstoft MS, Matthews RT, Digby GJ, Møller CL, Svendsen B, Gribble F, Reimann F, Holst JJ *et al*.: The melanocortin-4 receptor is expressed in enteroendocrine L cells and can regulate the release of peptide YY and glucagon-like peptide 1 *in vivo*. *Cell Metab* 2014, **20**:1018-1029. The first description of peripheral MC4R activity, that is basolaterally-targeted, involves L cell PYY and GLP-1 release and slows colonic transit.

25. Cox HM, Tough IR: Neuropeptide Y Y_1, Y_2 and Y_4 receptors mediate Y agonist responses in isolated human colon mucosa. *Br J Pharmacol* 2002, **135**:1505-1512.

26. Hyland NP, Sjöberg F, Tough IR, Herzog H, Cox HM: Functional consequences of neuropeptide Y Y_2 rceptor knockout and Y_2 antagonism in mouse and human colonic tissues. *Br J Pharmacol* 2003, **139**:863-871.

27. Tough IR, Holliday ND, Cox HM: Y_4 receptors mediate the inhibitory responses of pancreatic polypeptide in human and mouse colon mucosa. *J Pharmacol Exp Therap* 2006, **319**:20-30.

28. Tough IR, Forbes S, Tolhurst R, Ellis M, Herzog H, Bornstein JC & Cox HM: Endogenous peptide YY and neuropeptide Y inhibit colonic ion transport, contractility and transit by differentially via Y_1 and Y_2 receptors. *Br J Pharmacol* 2011, **164**:471-484.

29. Cox HM: Peptide YY: a neuroendocrine neighbor of note. *Peptides* 2007, **28**:345-351.

30. * Patel S, Mace OJ, Tough IR, White J, Cock TA, Warpman Berglund U, Schindler M, Cox HM: Gastro-intestinal hormonal responses upon GPR119 activation in lean and diseased rodent models of type 2 diabetes. *Int J Obesity* 2014, **38**:1365-1373. First study showing GPR119 signalling is present in gut mucosae from T2D rodents.

31. Tough IR, Cox HM: Glucagon-like peptide-1 receptor-mediated increases in short-circuit current are dependent on calcitonin gene-related peptide receptor activation in mouse ascending colon mucosa. *pA2 online* 2015, http://www.pa2online.org/abstracts/Vol13Issue3abst249P.pdf .

32. * Richards P, Parker HE, Adriaenssens AE, Hodgson JM, Cork SC, Trapp S, Gribble FM, Reimann F: Identification and characterisation of GLP-1 receptor-expressing cells using a new transgenic mouse model. *Diabetes* 2014, **63**:1224-1233. Important resolution of GLP-1 receptor expression in a reporter mouse, showing within the gut proximity between a GLP-1-containing L cell and a GLP-1 receptor-expressing submucosal nerve fibre.

33. Wang L, Goucerol G, Yuan PQ, Wu SV, Million M, Larauche M, Tache Y: Peripheral PYY inhibits propulsive colonic motor function through Y2 receptor in conscious mice. *Am J Physiol* 2010, **298**:G45-G56.

34. Forbes S, Cox HM: Peptide YY, neuropeptide Y and corticotrophin-releasing factor modulate gastrointestinal motility and food intake during acute stress. *Neurogastroenterol Motil* 2014, **26**:1605-1614.

35. Hansen KB, Rosenkilde MM, Knop FK, Wellner N, Diep TA, Rehfeld JF, Andersen UB, Holst JJ, Hansen HS: 2-Oleoyl glycerol is a GPR119 agonist and signals GLP-1 release in humans. *J Clin Endocrinol Metab* 2011, **96**:E1409-E1417.

36. Hansen HS, Rosenkilde MM, Holst JJ, Schwartz TW: GPR119 as a fat sensor. *Trends in Pharmacol Sci* 2012, **33**:374-381.

37. * Breton J, Tennoune N, Lucas N, Francios M, Legrand R, Jacquemot J, Goichon A, Guerin C, Peltier J, Pestel-Caron P, *et al*.: Gut commensal *E. coli* proteins activate host satiety pathways following nutrient-induced bacterial growth. *Cell Metab* 2016, **23:**324-334. Colonic infusion of bacterial proteins (one of which is anti-genically similar to α-MSH) differentially increase GLP-1 or PYY release and can alter host feeding behaviour.

38. Moss CE, Glass LL, Diakogiannaki E, Pais R, Lenaghan C, Smith DM, Wedin M, Bohlooly-Y M, Gribble FM, Reimann F: Lipid derivatives activate GPR119 and trigger GLP-1 secretion in primary murine L-cells. *Peptides* 2016, **77**:16-20.

39. Pedersen J, Ugleholdt RK, Jorgensen SM, Windelov JA, Grunddal KV, Schwartz TW, Fuchtbauer EM, Poulsen SS, Holst PJ, Holst JJ: Glucose metabolism is altered after loss of L cells and α-cells but not influenced by loss of K cells. *Am J Physiol Endocrinol Metab* 2013, **304**:E60-E73.

40. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, Tsujimoto G: Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med* 2005, **11**:90-94.

41. Milligan G, Alvarez-Curto E, Watterson KR, Ulven T, Hudson BD: Characterising pharmacological ligands to study the long-chain fatty acid receptors GPR40/FFA1 and GPR120/FFA4. *Br J Pharmacol* 2015, **172**:3254-3265.

42. Habib AM, Richards P, Rogers GJ, Gribble FM: Co-localisation and secretion of GLP-1 and PYY from primary cultured human L cells. *Diabetologia* 2013, **56**:1413-1416.

43. Edfalk S, Steneberg P, Edlund H: GPR40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes* 2008, **57**:2280-2287.

44. Christensen LW, Kuhre RE, Janus C, Svendsen B, Holst JJ: Vascular, but not luminal, activation of FFAR1 (GPR40) stimulates GLP-1 secretion from isolated perfused rat small intestine. *Physiol Reports* 2015, **3**:e12551.

45. * Hauge M, Vestmar MA, Husted AS, Ekberg JP, Wright MJ, Di Salvo J, Weinglass AB, Engelstoft MS, Madsen AN, Luckmann M *et al*.: GPR40 (FFAR1) - Combined Gs and Gq signaling *in vitro* is associated with robust incretin secretagogue action *ex vivo* and *in vivo*. *Mol Metab* 2014, **4**:3-14. The first description of biased signalling and enhanced efficacy for FFA1 ligands some of which are capable of co-coupling via G_q- and G_s-pathways.

46. Li Y, Kokrashvili Z, Mosinger B, Margolskee RF: Gustducin couples fatty acid receptors to GLP-1 release in colon. *Am J Physiol Endocrinol Metab* 2013, **67**:E651-E660.

47. Tough IR, Cox HM: Phenylthiocarbamide modulates ion secretion via release of PYY and 5-HT in mouse descending colon mucosa. *Gastroenterol* 2009, **136**:(5) A-235, S1615.

48. Brighton CA, Rievaj J, Kuhre RE, Glass LL, Schoojans K, Holst JJ, Gribble FM, Reimann F: Bile acids trigger GLP-1 release predominantly by accessing basolaterally-located G-protein coupled bile acid receptors. *Endocrinol* 2015, **156**:3961-3970.

49. Ullmer C, Alvarez-Sanchez R, Sprecher U, Raab S, Mattei P, Dehmlow H, Sewing S, Iglesias A, Beauchamp J, Conde-Knape K: Systemic bile acid sensing by G protein-coupled bile acid receptor 1 (GPBAR1) promotes PYY and GLP-1 release. *Br J Pharmacol* 2013, **169**:671-684.

50. Poole DP, Godfrey C, Cattaruzza F, Cottrell GS, Kirkland JG, Pelayo JC, Bunnett NW, Corvera CU: Expression and function of the bile acid receptor GpBAR1 (TGR5) in the murine enteric nervous system. *Neurogastroenterol Motil* 2010, **22**:814-825.

51. ** Dey N, Wagner VE, Blanton LV, Cheng J, Fontana L, Haque R, Ahmed T, Gordon JI: Regulators of gut motility revealed by a gnotobiotic model of diet-microbiome interactions related to travel. *Cell* 2015, **163**:95-107. This notable study used gnotobiotic mice to establish altered gut motility phenotypes linked to acute dietary change. Tumeric was shown to slow full-length gut transit in mice with a human Bangladeshi microbiota/diet; this involving BA secretion, deconjugation and enteric neuron Ret-signalling.

52. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM: SCFAs stimulate GLP-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* 2012, **61**:364-371.

53. Nøhr MK, Pedersen MH, Gille A, Egerod KL, Engelstoft MS, Husted AS, Sichlau RM, Grunddal KV, Poulsen SS, Han S et al.: *et al*. GPR41/FFAR3 abd GPR43/FFAR2 as cosensors for SCFAs in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinol* 2013, **154**:3552-3564.

54. Petersen N, Reimann F, Bartfled S, Farin HF, Ringnalda FC, Vries RGJ, van den Brink S, Clevers H, Gribble FM, de Konig EJP: Generation of L cells in mouse and human small intestine organoids. *Diabetes* 2014, **63**:410-420.

55. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M *et al*.: *Proc Nat Acad Sci* 2008, **105**:16767-16772.

56. * Forbes S, Stafford S, Coope G, Heffron H, Real K, Newman R, Davenport R, Barnes M, Grosse J, Cox HM: Selective FFA2 agonism acts via intestinal PYY to reduce transit and food intake, but does not improve glucose tolerance in mouse models. *Diabetes* 2015, **64**:3763-3771. The first characterisation of a selective FFA2 agonist showing PYY, rather than GLP-1 mechanisms in rodents.

57. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS: The microbial metabolites, SCFAs, regulate colonic T_{reg} cell homeostasis: *Science* 2013, **341**:569-573.

58. Macia L, Tan J, Vierira AT, Leach K, Stanley D, Luong S, MaruyaM, McKenzie CI, Hijikata A, Wong K.: *et al*. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammosome. *Nat Commun* 2015, **6**:6734-6747.

59. ** De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zituon C, Duchampt A, Backhed F, Mithieux G: Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* 2014, **156:**84-96. An important study revealing a causal relationship between SCFAs and intestinal gluconeogenesis that involves FFA3 in a gut-brain neural circuit, in normal and insulin-resistant conditions.

60. Psichas A, Sleeth ML, Murphy KG, Brooks L, Bewick GA, Hanyaloglu AC, Ghatei MA, Bloom SR, Frost G: The SCFA propionate stimulates GLP-1 and PYY secretion via FFA2 in rodents. *Int J Obes* 2015, **39**:424-429.

61. * Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK, MacDougall K, Preston T, Tedford C, Finlayson GS, *et al*.: Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* 2014, **64**:1744-1754. First-in-human study showing intracolonic propionate enhances PYY and GLP-1 release acutely and, longer-term reduces weight gain and adiposity in the overweight group.

62. Greenfield JR, Farooqi IS, Keogh JM, Henning E, Habib AM, Blackwood A, Reimann F, Holst JJ, Gribble FM: Oral glutamine increases circulating glucagon-like peptide 1, glucagon and insulin concentrations in lean, obese, and type 2 diabetic subjects. *Am J Clin Nutr* 2009, **89**:106-113.

63. Tolhurst G, Zheng Y, Parker HE, Habib AM, Reimann F, Gribble FM: Glutamine triggers and potentiates glucagon-like peptide-1 secretion by raising cytosolic Ca^{2+} and cAMP. *Endocrinol* 2011, **152**: 405-413.

64. Alamshah A, McGavigan AK, Spreckley E, Kinsey-Jones JS, Amin A, Tough IR, O'Hara HC, Moolla A, Banks K, France R *et al*.: L-arginine promotes gut hormone release and reduces food intake in rodents. *Diabetes Obes & Metab* 2016, **18**:508-518.

65. Joshi S, Tough IR, Cox HM: Endogenous PYY and GLP-1 mediate L-glutamine responses in intestinal mucosa. *Br J Pharmacol* 2013, **170**:1092-1101.

66. Diakogiannaki E, Pais R, Tolhurst G, Parker HE, Horscroft J, Rauscher B, Zietek T, Daniel H, Gribble FM, Reimann F: Oligopeptides stimulate GLP-1 secretion in mice through proton-coupled uptake and the calcium-sensing receptor. *Diabetologia* 2013, **56**:2688-2696.

67. Pais R, Gribble FM, Reimann F: Signalling pathways involved in the detection of peptones by murine small intestinal enteroendocrine L-cells. *Peptides* 2016, **77**:9-15.